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Final Technical Report

NAG 10 - 00801

ZOOLOGICAL EFFECTS OF VARIATIONS IN  
ATMOSPHERIC OXYGEN LEVELS



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June 1982

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(NASA-CR-169020) ZOOLOGICAL EFFECTS OF  
VARIATIONS IN ATMOSPHERIC OXYGEN LEVELS  
Final Technical Report (Kentucky State  
Univ., Frankfort.) 25 p HC A02/MF A01

N82-26954

Unclas  
CSCL 06B G3/51 28060

## INTRODUCTION

Originally, this project was begun at Kennedy Space Center in what was then called the Materials Testing Laboratory. John Gayle, who supervised that group, had asked a question which went something like "...is a normal sea level atmosphere best for life and how will determining that affect the space program?" They had begun exposing the fruit fly to various concentrations of oxygen at standard pressure. They had collected some survivorship data but were having some problems handling the research animals. The principal investigator learned of this project through efforts of the Minority Affairs Office (NASA) and their attempts to fund NASA related research in minority schools. Discussion ensued and by mutual agreement it was proposed to allow Kentucky State University to take over the work.

At Kentucky State University the project was expanded such that the role of certain gene enzymes in survival in modified atmospheres was to be examined along with a study of chromosome morphology as it related to these parameters in addition to the measurement of mortality and life span. In other words, it was to be a study in population genetics. Equipment to deliver various gas mixtures to the flies was not available on the market, and so it had to be designed and fabricated in the Materials Testing Laboratory. To study the gene enzymes a technique called starch gel electrophoresis was needed. Equipment and supplies for this work and the study of chromosome morphology was available on the market, although some of the equipment was made in the Materials Testing Laboratory to save the project money.

Even though the funding periods extended from May to May of each year, reporting generally went from December to December, because the individuals involved in the project reviewed the work and began preparation of a proposal for additional funding over the academic break at Christmas time. It must

be remembered that the investigators had only one half time released for research during the normal academic year and that some work had to be done during such break periods. The process of getting comments and/or suggestions from the support people at Kennedy Space Center, of getting the proposal to the appropriate people at Kentucky State University, and of getting the proposal through the "channels" at Kennedy Space Center and the University Affairs Office in Washington usually took until May of the following year. Therefore, for the most part, the review here will be based upon the calendar year.

#### RELEVANCE TO SPACE PROGRAM

As mentioned this project was begun to answer a somewhat rhetorical question about the suitability of atmospheric alternatives on life. Even then and especially now this type of research is exceedingly relevant to the future of space. Space transportation will offer important new opportunities in the utilization of the space environment for scientific research, manufacturing in zero gravity, and construction of large structures which may be used for greatly expanded communications, power generations or earth survey facilities (Smith and Jackson, 1978). Large space structures are envisioned for space-based operations, lunar bases, and space industrialization. In the evolution of these developments projected by Disher in 1978, a habitability module for long duration missions will be a must. Smith and Jackson believe that when the habitability module is added, a complete closure of the life support loops will become economically mandatory.

The need for ecologically closed systems was also emphasized recently by Winter in the opening address of the 1978 AIAA/ASMA meetings in New Orleans and to me in personal communication following that address. Similarly, Popma and Spurlock (1978) insisted that the open mode system currently being

used must be replaced with some form of closed mode CELSS (closed ecology life support system).

Current theories in ecosystem strategies and evolution indicate that stability is enhanced with relatively high species diversity ratios (see for example Odum, 1969). It will probably prove necessary to include a number of species into the system which do not provide essentials directly to the human inhabitants, but rather facilitate the stable and continued recycling of essential nutrients and wastes through the entire system. For example, carrion beetles and other similar insects greatly facilitate the turnover of nutrients by reducing dead animal material to a size and form which is more easily utilized by decomposing bacteria. Similarly termites and many forms of formicidae (ants) process dead plant matter. Importantly then, high priority should be placed on finding the types of organisms which are needed to interact in a CELSS system and the relative diversity such a system should have. Before then, however, the long term effects of space cabin/space station environments must be established.

The total air pressure in future CELSS environments has not been determined at the present time. A normal sea level atmosphere would be ideal, but those projecting CELSS conditions believe that some pressure reduction will be mandatory. In 1967, E.M. Roth explained that at pressures below 5 to 7 psi (250 to 500 torr) the weight of a cabin wall is independent of the pressure and that above 7 psi the weight penalty caused by reinforcement rises steeply with increasing pressure. In 1977, Thomas Heppenheimer presented a review of the literature and thinking which existed at that juncture and came to the same conclusion. A similar review (O'Neill, 1978) recently supported that view.

At the 1978 AIAA/ASMA meetings in New Orleans, J.K. Jackson explained to

me that while the crew module and spacelab system of Space Shuttle will be at 14 psi, the EVA suits are still maintained at 4 psi. Because of this a prebreathing period of 15 minutes or more is necessary prior to EVA. Although development of a standard pressure suit is underway, it was his opinion that in the future a drop to 10 psi with oxygen enrichment may be necessary to facilitate extravehicular travel, docking, etc. J. Billingham explained to me at that same meeting that the cost factor in space settlements can be cut drastically by lowering both air pressure and gravity. He envisioned a system at one half normal air pressure and 0.35g.

A recent design study for Space Settlements conducted by NASA and the American Society for Engineering Education which was directed by the Ames Research Center (NASA) came to the conclusion that a space settlement should be a one half normal air pressure with a mix of about 50% nitrogen/50% oxygen (more or less 200 torr of each).

Excessive oxygen pressure has long been known to be toxic but only a few studies are available which examined zoological effects of oxygen pressures between 200 and 760 torr in 760 torr pressure on Drosophila and other small invertebrate organisms. Studies by W.O. Fenn and his coworkers (see especially Fenn, W.O., 1967) and Philpott and his colleagues at the Ames Research Center, NASA, (see especially Philpott et al, 1974) and by us, have shown that moderate elevations of oxygen pressure can be toxic under some conditions and not under others. For example, Philpott demonstrated that 250 torr pure oxygen is toxic to Drosophila, and yet we showed that tensions as great as 300 torr are not toxic to Drosophila if a diluent gas (nitrogen) is mixed in the atmosphere at pressures around 450 torr. This is a strong indication that the presence of a diluent gas is an important factor in the toxicity of oxygen.

It seems apparent that a knowledge of the atmospheric extremes or total pressures and relative oxygen concentration in which small life forms can survive and reproduce is important and may in fact be a determining factor in the final selection of the future atmospheric mixes. Accordingly, investigations were begun in an attempt to identify safe atmospheric alternatives.

#### ACTIVITIES AND ACCOMPLISHMENTS

Original proposal was accepted March 1974. No money was received until June 1974, so no equipment could be ordered until that time.

During the summer months, wild type fruit flies were collected from the Frankfort area. Taken from two different localities and three different times, three stocks were established and maintained in the laboratory. A special strain of vestigial wing and brown-eyed flies was also cultured for use. Two species of Tribolium were obtained from a biological supply house and maintained in the laboratory. Scientists from KSC's Materials Testing Laboratory and biologists from KSU designed a system to maintain the flies in the desired atmospheres. A manifold system was designed which would deliver the desired gas to separate culture vials.

The equipment and materials supplied by Kennedy Space Center arrived July 15, 1974. It was August before the equipment was set up and the laboratory was ready to begin even preliminary work. Once experimental trials were begun it was immediately evident that the flow system did not distribute gas evenly to all the culture bottles. The uneven gas distribution created a problem with humidity regulation. Various modifications were attempted to solve the humidity and gas flow problems.

Following the preliminary trials to alleviate the gas distribution problems, a series of seventeen culture vials, each with fifty flies, were maintained on the oxygen flow system for sixty days from October 28 to December 26, 1974. Vials of vestigial wing and wild type fruit flies were maintained at differing oxygen concentration levels, from 10 to 50% O<sub>2</sub>.

Mortality rates were found to be greater at 50% O<sub>2</sub>, but there was not a mortality increase between 10 and 40% O<sub>2</sub>. At this time we believed failure to keep constant temperature masked the effect of variant oxygen concentrations except at the 50 percent extreme.

Generally we made the following tentative conclusions: 1) vestigial wing flies have greater mortality rates in any oxygen concentration than the wild type do, 2) the males of both types exhibit greater mortality rates than the females do at any given concentration, and 3) as a population, the flies living in 50 percent oxygen have greater mortality rates than flies living at any lower oxygen concentration.

During the summer of 1974 we had an emergence of a brood of the periodical 17 year cicada take place in Kentucky. These species are of special interest to biologists because of their unusual life cycle and mixed species broods. A study of the genic frequency within and between species in the Frankfort area was quickly and easily done with the equipment and supplies at hand. This work was published in 1978 (Ralin and Kloek, 1978).

By December 26, 1974, twelve enzyme gene loci identifiable by starch gel electrophoresis were examined in each of the three wild type populations and the vestigial wing flies. The first gel was run September 20. By October 24 techniques were refined and the staff sufficiently trained to begin large scale examination of the populations. Scorable patterns were found for the following enzymes: alcohol dehydrogenase or ADH (2 loci, Adh 1, and Adh 2),



glutamate oxaloacetate transaminase or GOT (two loci, GOT 1, GOT 2), isocitrate dehydrogenase or IDH (one locus, Idh), malate dehydrogenase or MDH (2 loci, Mdh-NAD, Mdh-NADP), malic enzyme or ME (1 locus, Me), aldolase or ALD (1 locus, Ald), phosphoglucumutase or PGM (1 locus, Pgm), alpha glycerophosphate dehydrogenase or  $\alpha$  GPDH (1 locus,  $\alpha$  gpdh), and 6-phosphogluconic dehydrogenase or 6-PGDH (1 locus, 6-pgdh). These twelve loci were run on approximately 800 individual flies.

The vestigial wing, brown eyed stock proved to be extremely homozygous in that each locus was fixed for the most common allele in the wild type strains. In the wild type, six loci gave evidence of no allelic segregation. They were Got 1 and 2, Mdh-NAD, Mdh-NADP, Me, and Ald. Five loci proved to be segregating for two alleles in high enough frequencies so that homozygotes for the rarer allele were expected and observed. They were: Adh 1, Adh 2, 1 dh,  $\alpha$ -gpdh and 6-pgdh. Pgm proved to be segregating for three alleles, although frequencies of the rarer alleles were not high enough to expect or observe homozygotes. All six loci fit reasonably well with Hardy-Weinberg expectations. Larva as well as adults were examined for ADH, MDH, ME, IDH, and GOT. Of these there appear to be quantitative differences between larva and adult ADH 2 and both MDH's.

Within six months then, the following was accomplished: 1) the fly stocks were established, 2) the equipment was put into operation, 3) the genetic make-up of the flies was established, and 4) a preliminary indication that oxygen concentrations in excess of 50% were toxic to the flies.

Work during 1975 involved single generation trials in various oxygen/nitrogen mixes. Mixes of 9%, 20%, 33% and 49% oxygen at standard pressure were examined in most detail. In this second year some experimental work also went into improving stability in temperature and gas concentration. The

Materials Testing Laboratory designed plexiglas cages that would allow distribution of gas to large numbers of flies at once. Now the entire population could be exposed in one single chamber, rather than in smaller numbers in separate bottles. The simple survivorship studies of 1974 were expanded to include several population parameters which could be generated from the data being collected.

Adult survivorship was calculated and expressed as the probability at eclosion of being alive at age  $x$  and is designated  $l_x$  ( $l_0=1$ ). Life expectancy was calculated from the relationship,  $e_x = \left[ \sum_{x}^n l_x + l_{x+1}/2 \right] / l_0$ . Egg laying rates per female per five day count period were determined from five hour egg laying periods and designated  $m_x^e$ . A net reproductive rate of eggs per female per lifetime is calculated from the expression  $\sum_x l_x m_x^e$  and is designated  $R_0^e$ . The counts of eclosing flies from the egg bottles allows the determination of hatching percent at each age interval. By multiplying each age specific egg laying rate by the hatching percentage for each age class,  $m_x^a$  (adult flies produced per female per lifetime) was calculated and with it a net reproductive rate based upon adult individuals was produced, designated  $R_0^a$ . Significance between survivorship rates was determined with a simple analysis of variance using an F ratio comparing within versus between treatments. Individual tests were applied in those instances where the overall F ratio proved significant. The 5% confidence level was used in all cases.

The work with gene enzymes established that enzymes known to denature in vitro in high oxygen concentrations did not denature in vivo in high oxygen concentrations. This in itself was noteworthy. Further, during the year a comparison of the relative occurrence of the various forms of these enzymes between oxygen treated flies and control flies was done. The results of the first two years work was published (Kloek et al., 1976).

One of the student workers, Terry Kelly, suggested a further expansion during this year. He suggested that examination of sectioned, paraffin embedded flies might provide a diagnosis of oxygen poisoning. Drosophila has been a common organism with which to examine the effects of aging. The histological effects of oxygen poisoning seem to be the same as the effects of normal aging in Drosophila. Much of this work was conducted at the Ames Research Center at Moffett Field, California. Because the experimental conditions in our laboratory were not directly comparable with the work of others, we decided in 1975 to attempt the same comparisons as the Ames Center. Jaime Miquel from the Ames Center had encouraged our work and Dr. Miquel requested some of our specially treated flies for his studies. Preliminary comparisons of nervous and adipose tissue types were made.

Data from the work in 1975 raised some interesting questions that were not originally anticipated in the project. For example, 100% oxygen at 1/3 normal atmosphere shortened life span in Drosophila (Philpott et al., 1974), but our flies maintained at 33% oxygen and 67% nitrogen at normal pressures showed no significant change in life span compared with flies reared in a normal atmosphere. As a result of this observation, scientists at NASA and KSU began considering the possibility of reduced pressure studies in the project's future work.

Late in January 1976, work was begun with multiple generation trials. Previous work had established that different gene enzymes were favored in different oxygen concentrations, that is, flies surviving high oxygen concentration had different gene frequencies than flies surviving low oxygen concentrations. This suggested that flies having certain combinations of gene enzymes were better equipped to survive certain conditions than others. The survivors would reproduce and pass their fitness to their offspring, hence survival of the fittest. This implied that following a period of time, a

reproducing population could adapt or evolve to survive in atmospheric conditions which originally were not fit for life. We believed that 10 generation trials would be adequate to determine if adaptation from generation to generation occurred over time.

Gas flow rates and other life support conditions that were used were determined experimentally during the previous autumn. As the generations continued it became evident that the flow rates which had maintained the desired experimental conditions were no longer adequate. In the fifth generation the trials were terminated. Close examination of the plexiglas population cages revealed that the lids had warped considerably, the hinge mechanism had developed leaks and that the bonding agent in some of the seams had separated sufficiently to allow gas leaks. Field repairs were initiated and following pressure tests with smoke and carbon dioxide vapors the cages were deemed sufficiently tight to allow a resumption of trials using flow rates slow enough to maintain the desired atmosphere and yet provide reasonable conservation of gas.

Cohort populations of D. melanogaster numbering 1,000 or more were maintained in concentrations of 8%, 21% and 50% oxygen. Each was begun from a stock captured in the wild and maintained in the lab. At the end of 1976 each had been living and reproducing in its assigned atmosphere for seven generations. We monitored vital population parameters such as fecundity, fertility, and larval and adult survivorship. We also sought a correlation with allelic frequencies in gene enzymes concerned with oxygen metabolism as we did in Klock et al. (1976a). We found changes in gene frequencies and survival rates in the seven generation flies, but didn't prepare formal analysis until the tenth generation was reached (in the next year of funding).

We continued our histological examination of nerve tissue in oxygen

treated flies. Vacuoles, known to appear in aged flies, appear in oxygen treated flies long before they can be considered "old aged." We needed repeatable data before we could draw any general conclusions from this work, however.

Equipment and supplies obtained with a small supplemental grant were used to help Kennedy Space Center investigate a disease attacking Florida citrus groves. The idea was to determine if trees which survived the disease were genetically different. Since we had established that flies surviving high oxygen concentrations were genetically different than flies living in lower concentrations, it seemed logical to see if there was also a genetic difference between the diseased plants and those which had survived the disease. This was done at the request of Dr. John Gayle. We found diseased and healthy plants to be identical at 8 enzyme loci. This project was not carried any further. During this year a second paper was published (Kloek et al., 1976b).

During 1977 the multiple generation trials were repeated with special attention being given to 8%, 21%, and 50% oxygen. Our replications confirmed that survivorship, life span, and fecundity will improve from generation to generation in oxygen concentrations which initially cause a reduction in these parameters. These results were conjoined with other multigeneration work and published in 1979 (Kloek et al., 1979a).

In August 1977 we began our first trials with hypobaric oxygen enriched atmospheres. Even though much information is available concerning the effects of hyperbaric concentrations of oxygen and nitrogen, less is known about the effects of abnormal pressures of nitrogen on concentrations of oxygen between 0.21 ATM and 1.0 ATM.

We found that survivorship in a 50/50 oxygen/nitrogen mix at 300 torr total pressure is identical to survivorship in normal atmosphere. This work

showed that a fairly drastic reduction in atmospheric nitrogen did not create or increase a toxicity problem with partial pressures of oxygen found in normal air. It also reaffirmed that overall pressure reduction from 760 torr to 300 torr did not create survivorship problems which might stem from factors other than those related to the respiration of oxygen. These results established a baseline or platform from which we began to look further into the relationships of enriched oxygen low pressure atmospheres near 300 torr total pressure.

The multiple generation work at standard pressure continued in 1978. Early in this year a new set of trials using 60% oxygen/40% nitrogen was begun. Ten more generations consumed most of that year. Manuscript preparation of the multiple generation trials was completed and it was submitted for publication late in the year (Kloek et al, 1979a).

The effects of temperature on the experimental parameters were also begun during the year. Temperature had long been known to affect life span, fecundity, etc., in "cold blooded" organisms such as the fruit fly. Since the extremes of survivorship were being pretty well determined by our work at one temperature (26°C), it seemed logical to repeat the experiments in at least two other temperatures and accordingly establish a range of temperature tolerances as well as tolerances of oxygen concentration. Moreover, it would be important to see if any synergistic effects existed between temperature and pressure. The temperature extremes of 18°C and 32°C were initially chosen because they were near the known extremes for the flies which live in a normal sea level atmosphere.

The results were pretty much as expected, that is, no strong synergistic effects between temperature and oxygen concentration were found, and that life span was longer in lower temperatures and shortened at higher temperatures.

The extreme limits of oxygen concentration tolerable for life and the effect on the other population parameters were not established in these first trials. This work was published with additional work in this area in 1979 (Kloek, 1979).

During this time, trials were run at a reduced pressure of 300 torr. Preliminary results of the hypobaric studies were published (Kloek, 1978). We found from our early work in this area that flies living at 26°C in concentrations as great as 60% oxygen (150 torr partial pressure, 300 torr total pressure) had survivorship curves similar to flies living in normal air at 26°C. Reproductive parameters were not quantified, but appeared to be near normal. In June we had a failure of the motor in the hypobaric unit. The unit was repaired as soon as parts were received. It was again in operation in August.

During the summer of 1978, Dr. Charles Bennett conducted a study of the effect of high oxygen concentrations on the cardiovascular system in rats. A report of his work is included in the publications.

In 1979 we were looking for extremes of temperature, total pressure and oxygen concentration in which successful reproduction, survivorship and population adaptation could occur. These were the main factors which we had found to be important in establishing atmospheric limits for population survival of poikilothermic organisms. In this year we also repeated many of the trials in which we had drawn no firm conclusions or needed more data.

The following chart contains the life expectancies ( $e_x$ ) and reproduction rates ( $R_0$ ) drawn from all of the work completed up to December of 1979.

Except for the special study conducted in 1978 by Ms. Martha Woelfel as a summer participant, the vacuolation data was not developed as a single unit. Rather, embedded flies were stored and selected exposures were examined in instances where oxygen toxicity was suspected but not clearly evident. For example, flies in 100% oxygen in the reduced pressure atmosphere have normal longevity but appeared to have reduced reproduction (data were still being worked up). We sectioned those which had been exposed to this atmosphere and found extensive vacuolation and ruled on this basis that this atmospheric alternative was toxic. Below find a brief summary of the work completed through 1979.

Experimental Conditions*			Brain Vacuolation
26°	20%	1 ATM.	A little vacuolation at 50d. Maximum life span 60d.
26°	8%	1 ATM.	None fixed.
26°	33%	1 ATM.	Same as 20%.
26°	45%	1 ATM.	30d normal. 40d many vacuoles seen.
26°	50%	1 ATM.	30d normal. 40d many vacuoles seen.
26°	55%	1 ATM.	21d normal. 21d-30d many vacuoles. 30d all dead.
26°	60%	1 ATM.	Not finished.
18°	8%	1 ATM.	Not finished.
18°	20%	1 ATM.	At 105d-110d and 121d a few small scattered vacuoles seen. More at 126d. Even at 131d still not as many as in 54d control fly.
18°	50%	1 ATM.	Not finished.
18°	60%	1 ATM.	Vacuoles first seen at 31d though not many. More at 40d.
26°	33%	1/3 ATM.	Not finished.
26°	20%	1/3 ATM.	Vacuoles seen at 25d. Need more work on these.
26°	60%	1/3 ATM.	A few small vacuoles seen at 39d. At 54d and 61d still not many vacuoles.
26°	80%	1/3 ATM.	80-VC-1 Vacuoles first seen at 49d. A 54d fly brain had about the same number of vacuoles as a 54d brain of control flies. In 80-VC-2 a few vacuoles in 34 and 39d flies.
26°	100%	1/3 ATM.	Vacuoles first seen at 25d. Many at 35d. Many and large at 46 and 50d.

\* All single generation trials.



Experimental Conditions	#Trials and #Flies	Life Expectancy $e_x$ in days	Reproductive Rate $R_0$
Standard Pressure $26 \pm 2^\circ\text{C}$			
20% $\text{O}_2$	8 trials, 2,000 flies	$37 \pm 1.4$ SE	$1,777 \pm 200$ SE
50% $\text{O}_2$	1 trial, 250 flies	26.5	881
50% $\text{O}_2$	5 trials	$35.6 \pm 2.4$ SE	$1,117 \pm 79$ SE
60% $\text{O}_2$	14 trials, 3,500 flies	$19.3 \pm 1.8$ SE	
	4 trials, 1,000 flies		$588 \pm 145$ SE
6% $\text{O}_2$	1 trial, 250 flies	40+	
8% $\text{O}_2$	16 trials, 4,000 flies	$37 \pm 1.0$ SE	
33% $\text{O}_2$	1 trial, 250 flies	47	
	1 trial, 100 flies	41	
$18 \pm 1^\circ\text{C}$			
20% $\text{O}_2$	4 trials, 1,000 flies	$68 \pm 1.9$ SE	
	1 trial, 250 flies		1,265
60% $\text{O}_2$	5 trials, 1,250 flies	$30 \pm 2$ SE	
	1 trial, 250 flies		871
8% $\text{O}_2$	2 trials, 500 flies	58	
250 torr Pressure $26 \pm 2^\circ\text{C}$			
20% - 50 torr	1 trial, 250 flies	10	
33% - 85 torr	1 trial, 250 flies	39	2,287
60% - 150 torr	2 trials, 500 flies	42	
80% - 200 torr	2 trials, 500 flies	42	
	1 trial, 250 flies		2,404
100% - 250 torr	2 trials, 500 flies	41	

In May, 1979, Gerrit Kloek presented a paper at the 1979 Annual Scientific Meeting of the Aerospace Medical Association. The title was "Safe Extremes in Oxygen Concentration for Drosophila in Standard and Reduced Pressure Atmospheres." The paper was published in "Preprints of the Scientific Program, Aerospace Medical Association, 1979, Annual Scientific Meeting."

Much of our early work involved development of experimental procedures, equipment modifications and the establishment of experimental extremes beyond which further investigation was not necessary. These activities were conducted because this was essentially a pioneering study and there were no precedent studies to follow. Late in 1979 the progress was reviewed by Gerrit Kloek, the principal investigator, and William Knott and G. Wyckliffe Hoffler, the Technical Officers from Kennedy Space Center. Using the knowledge and experience gained from earlier work, a three dimensional experimental design was put forth which allowed an examination of any possible interactions of three parameters, total atmospheric pressure, oxygen partial pressure, and temperature.

This design encompassed 64 cells, each cell representing a combination of temperature, oxygen pressure and total pressure. Initially the temperature settings were 10°C, 18°C, 26°C and 34°C and the total pressures were 160 torr, 250 torr, 430 torr, and 760 torr. The oxygen partial pressures were left tentative at the outset. The continued survivorship and overall vitality of the flies was of primary concern. If reproduction and general vitality were normal or near normal, it was projected that the organisms would adequately exploit their niche. Accordingly, survivorship, expressed as percent survivorship at regular intervals, average life expectancy ( $e_x$ ), and the average reproductive rate ( $R_0$ ) were determined and reported.

In 1980 we attempted to quantify the histological vacuolation studies so that they could become another reportable parameter. Examination of several hundred slides showed that the progress of the vacuolation occurs in identifiable stages as the fly ages in elevated oxygen atmospheres. The stages were divided into five levels and each brain classified into one level depending upon the extent of vacuolation.

The levels were numbered zero (0) to four (4). Zero was a brain having no vacuoles. Level one had small vacuoles in the anteriormost portion of the protocerebrum where this effect first appeared. In level two, vacuoles appeared in the optic nerve and usually a few could be seen in the center of the protocerebrum in the region of the esophageal canal. In stage three, vacuoles could be found throughout the protocerebrum but the brain tissue remained intact. In stage four, the vacuolation was so extensive that the brain tissue appeared macerated.

Although selected sections were studied from all the trial sets conducted, only the 250 torr pressure set had been completed at the end of 1980.

Further examination of other trials in 1981 led us to change our classification criteria, but this work in 1980 became the base for all of our further vacuolation studies.

Drosophila melanogaster populations maintained in 30% to 60% oxygen at 250 torr total pressure were found to have survivorship curves, maximum life span, average life expectancy and reproductive rates which were similar to flies reared in normal sea level atmospheres. In addition, brain vacuolation advanced no farther than it does in these normal control flies. Accordingly, we projected that a total atmosphere of 250 torr, and a partial pressure of oxygen between 83 and 150 torr, is safe for continued survivorship and vitality. However, no multigeneration trials were run to support this belief in 1980.

We planned to run 18°C trials at 250 torr total pressure during this funded period but this required a second Freas 818 unit (cold incubator). Although the unit was ordered in April 1980 it was not received until late autumn 1980. Before we could use this incubator fittings had to be installed, again causing delays. The internal construction of this unit was different from our other Freas unit and it was early 1981 before this unit was in operation. We also had problems with leaks and early trials were terminated. It was July 1981 before all the bugs were worked out and the unit was put to use.

The proposal which was submitted in January of 1981 which requested additional funds for May 1981 to May 1982 was returned with a request to reduce the budget. Previously, the budget had been verbally approved by Kennedy Space Center and the University Affairs Office (NASA), but a changing political atmosphere in Washington precipitated a cut-back in funding. It was not until October 1981, that additional funding was provided, and then with the proviso that the project be terminated and all possible conclusions be drawn during the last twelve months of work. Work continued from May 1981 to October 1981 under a period of forbearance. No money was supplied from NASA during that time, but a reduced work schedule was maintained because residual funds were available. Below is a table of the work done during that period. Not all of the data are worked up, and as mentioned above, an objective of the last grant is to just that. These data and their conclusions will be presented in the final report of the current grant.

Date	250 torr	760 torr			
	Total Pressure	Total Pressure			
1/81	29° - 100%	29° - 20%			
2/81	26° - 100% P	29° - 10%	12° - 100%		
3/81	26° - 100% f <sub>1</sub>	29° - 60%	12° - 40%		
4/81	26° - 100% f <sub>2</sub>	29° - 80%	↓	29° - 60%	29° - 20%
5/81	26° - 100% f <sub>3</sub>	12° - 60%	29° - 40%	29° - 10%	
6/81	26° - 100% f <sub>4</sub>	↓	12° - 20%	12° - 40%	
7/81	18° - 60%	↓	↓	↓	26° - 20% 29° - 20%
8/81	↓		↓	↓	Repeated above
9/81	↓	29° - 100%	12° - 80% - 2 trials		29° - 80%
10/81				18° - 20%	Repeated above
	380 torr Total Pressure			↓	
11/81	26° - 40%	29° - 40%	29° - 20%	↓	Repeated above
12/81			29, 18° - 20%		
1/82	Repeated above	18, 19° - 20%	↓	26° - 100%	26° - 20%
2/82	26° - 80%	↓	↓	12° - 60%	↓
3/82	↓	18, 29° - 20%	↓	↓	Repeated above
4/82	26° - 20%	29, 18° - 20%	↓	↓	↓
5/82	↓	↓			

#### PARTICIPANTS

One of the goals of these special grants to minority schools was to provide small minority schools, their students and faculty, with exposure to research and to help build a research facility which the school might not otherwise be able to do. In this regard, I believe this grant has been extremely successful. Kentucky State University now has an air conditioned laboratory of about 1,000 square feet which has two Freas environmental chambers and a unique vacuum flow control console among many other pieces of common laboratory equipment. The facilities have been shared with other research projects and it is also used in basic instruction. The latter comes about in two ways. First, special problems for advanced students are conducted here and second, the lab is used as a demonstration of research techniques to the introductory student. Over the years, fifteen students have been supported by the NASA funds, 15 students which might not have had the opportunity to do so otherwise. Moreover, at least that many have been associated with the laboratory as so called "work study" students. These students receive support from other university sources to work and study. Because of this facility we are able to supervise at least one of these people each semester. Below find the fifteen students directly supported by this grant and a brief statement of what they did following employment.

1. Frank Bush, in industry
2. Shelia Morrow, transferred, lost contact
3. Tyrone Collins, graduate school, U of Cin.
4. George Sebron, undergraduate work
5. Danny Chacko, dental school, UL
6. Jerry Gibson, graduate school, LSU
7. Robert French, dental school, UK

8. Steve Kirchner, transferred, lost contact
9. Sushil Jain, optometry school, Southern
10. Terry Kelly, in industry
11. Alan Hammonds, in industry, State Crime Lab
12. Linda Mahoney, NASA research (Winkle)
13. Johnny Scott, optometry school, Southern
14. David Moore, medical school, UL
15. Deb Weidler, accept. graduate school, Purdue

Most of the students worked both their junior and senior years. Two of the students have transferred and we have lost contact with them. Of the remaining thirteen, one is still pursuing an undergraduate degree at KSU. Three are now working in industry. One is still working with the NASA project in the capacity of research scientist. Three of the students are involved in graduate work. One is at the University of Cincinnati; one is at Louisiana State University; and the third has been accepted into Purdue University's graduate program. Two of the former NASA students are attending dental school, one at the University of Kentucky and the other at the University of Louisville. Optometry school has attracted two of the students. One has graduated from Southern Optometry School and is serving in the Navy. The other has been accepted into Southern's program. One of the students is now pursuing a career in medicine at the University of Louisville's Medical School. Two of the students have coauthored publications with Dr. Klock on specific NASA research that they were involved in.

Five faculty and one staff member have worked in the project at one time or another. With title and a brief statement of participation find them listed below:

1. Dr. Gerrit Klock, Professor, one of the original grantees and project

director during eight years of the grant,

2. Dr. Dennis Ralin, Assistant Professor (at time of employment), one of the original grantees, participated three years, now Chairman, Department of Biology, Millikin University,
3. Dr. Gertrude Ridgel, Professor and Chairman of the Division of Science, Mathematics and Nursing, Kentucky State University, an original grantee, participated 18 months,
4. Dr. Charles Bennett, Assistant Professor, participated one summer,
5. Ms. Martha Woelfel, Assistant Professor, participated one summer,
6. Ms. Linda Winkle, Research Biologist, full time staff member and assistant director for four years.



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